PATENT
Docket No.: 20011/1371

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Beck et al.	Examiner: B. Coleman
Serial No.	:	09/704,306	
Cnfrm. No.	:	4919	Art Unit: 1624
Filed	:	November 2, 2000	
For	:	NOVEL 4-PHENYL SUBSTITUTED TETRAHYDROISOQUINOLINES AND USE THEREOF	

SECOND DECLARATION OF BRUCE F. MOLINO UNDER 37 C.F.R. § 1.132

I, BRUCE F. MOLINO, pursuant to 37 C.F.R. § 1.132, declare:

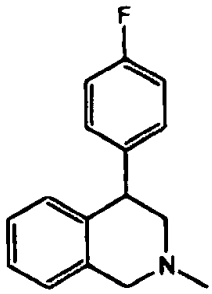
1. I received a B.S. degree in Chemistry from Rutgers University in 1977 and Ph.D, degree in Organic Chemistry from the University of Maryland in 1984.

2. I am the Senior Director of the Medicinal Chemistry Department at Albany Molecular Research, Inc.

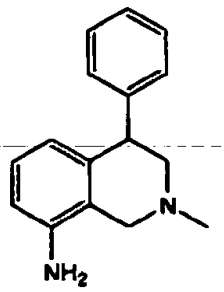
3. It is my understanding that Albany Molecular Research, Inc. is the assignee of the above patent application.

4. I present this declaration to demonstrate that compounds of the present application achieve greater potency for the norepinephrine transporter ("NET") and dopamine transporter ("DAT") than does compound 1 from Canadian Patent Application Serial No. 2,015,114 to Mondeshka et al. ("CA 2015114").

5. In particular, in addition to the compounds of the present invention (identified below as the PH-7032 compounds), the following compounds were tested:



reference CA 2015114
1



Nomifensine
2

6. In order to evaluate the relative affinity of the various compounds for NET, DAT, and SERT, HEK293E cell lines were developed to express each of the three human transporters. cDNAs containing the complete coding regions of each transporter were amplified by polymerase chain reaction from human brain libraries. The cDNAs contained in pCRII vectors were sequenced to verify their identity and then subcloned into an Epstein-Barr virus based expression plasmid (E Shen, et al., Gene 156:235-239 (1995)). This plasmid containing the coding sequence for one of the human transporters was transfected into HEK293E cells. Successful transfection was verified by the ability of known reuptake blockers to inhibit the uptake of tritiated norepinephrine, dopamine, or serotonin.

7. To test compounds for binding, cells were homogenized, centrifuged, and then resuspended in incubation buffer (50mM Tris, 120 mM NaCl, 5mM KCl, pH 7.4). The appropriate radioligand was then added--i.e. [^{3}H] Nisoxetine (86.0 Ci/mmol, NEN/DuPont) was added to a final concentration of approximately 5 nM (for NET binding), [^{3}H] WIN 35,428 (84.5 Ci/mmol) at 15 nM was added (for DAT binding), and [^{3}H] Citolapram (85.0 Ci/mmol) at 1 nM was added (for SERT binding). Various concentrations (10^{-5} to 10^{-11} M) of the compound of interest were then added to displace the radioligand. Incubation was carried out at room temperature for 1 hour in a 96 well plate. Following incubation, the plates were placed on a harvester and washed quickly 4 times with (50 mM

tris, 0.9% NaCl, pH 7.4) where the cell membranes containing the bound radioactive label were trapped on Whatman GF/B filters. Scintillation cocktail was added to the filters which were then counted in a Packard TopCount. Binding affinities of the compounds of interest were determined by non-linear curve regression using GraphPad Prism 2.01 software. Non-specific binding was determined by displacement with 10 micromolar mazindol. The results of these binding assays for the various compounds tested is set forth in Tables 1 and 2 below.

Table 1

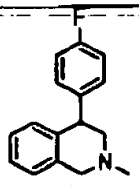
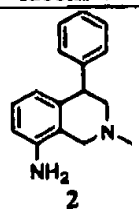
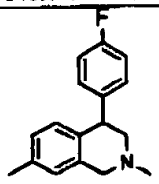
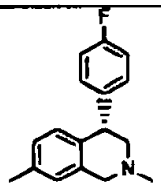
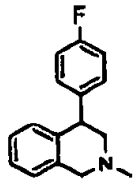
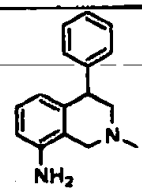
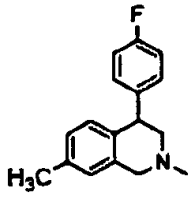
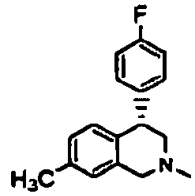
Compounds	NET, Ki nM	DAT, Ki nM	Selectivity DAT/NET
 1 racemate	52	256	4.9
 2 Nomifensine	23	72	2.25
 PH-7032 compound racemate	15	76.5	5.1
 PH-7032 compound (S)-(+)-enantiomer	7.1	36	5.1

Table 2

Compounds	NET, Ki nM	SERT, Ki nM	Selectivity SERT/NET
 1 reference CA 2015114	52	439	8.4
 2 Nomifensine	23	1036	45
 PH-7032 compound racemate	15	396	26.4
 PH-7032 compound (S)-(+)-enantiomer	7	231	33

8. The results in Table 1 demonstrate that compound 1 is approximately three-fold less potent for NET (based on the NET, Ki value) than the PH-7032 compound racemate and seven-fold less potent than the PH-7032 compound (S)-(+)-enantiomer shown in Table 1.

9. The results in Table 1 demonstrate that compound 1 is approximately threefold less potent for DAT (based on the DAT, Ki value) than the PH-7032 compound racemate and sevenfold less potent than the PH-7032 compound (S)-(+)-enantiomer shown in Table 1.

10. Compound 2, known as Nomifensine, has demonstrated efficacy in the treatment of depression (Brogden, R.N. et al *Drugs*, 18(1):1-24 (1979)) and attention deficit hyperactivity disorder in clinical trials (ADHD, Shekim, W.O. et al. *J. Nerv. Ment. Dis.*, 177:296 (1989)). The efficacy of this clinical agent is attributed to the potency and selectivity of Nomifensine for blocking the NET and the DAT. Nomifensine was tested separately in the same transporter assay with compound 1 and PH-7032 compounds. Comparison of the NET and DAT Ki values for Nomifensine with the PH-7032 compounds shows that the values are more closely matched by the PH-7032 racemate than the compound 1 racemate. Furthermore, the PH-7032 compound (S)-(+)-enantiomer possesses even better potency for NET and DAT than Nomifensine, the PH-7032 compound racemate, and compound 1. As a result of this unexpected increase in potency for blocking the NET and DAT, one would expect that the PH-7032 compounds, especially the (S)-(+)-enantiomer would be as potent or more efficacious than Nomifensine for the treatment of depression, ADHD and other CNS disorders where blocking transporter uptake is implicated. On the other hand, compound 1 would not be expected to be as potent nor as efficacious as Nomifensine or PH-7032.

11. The results in Table 2 demonstrate that compound 1 does not achieve a binding affinity for serotonin transporter protein to a binding affinity for norepinephrine transporter protein ratio of at least 20:1. Moreover, this compound is less selective for the NET vs. the SERT than compound 2, the PH-7032 compound racemate, or the PH-7032 compound (S)-(+)-enantiomer, based on ratios of the Ki values (SERT, Ki / NET, Ki). The class of drugs known as serotonin selective reuptake inhibitors ("SSRI"), exemplified by the antidepressant Prozac™, are associated with side effects like sexual dysfunction in many patients. The compounds of the present invention, like PH-7032 compound racemate or PH-7032 compound (S)-(+)-enantiomer, possess greater potency and selectivity for blocking the NET and DAT vs. the SERT and would be expected to possess fewer side effects due to the lack of effect on serotonin.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of

Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 12, 2003

Bruce F. Molino

Bruce F. Molino

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